

HYBRIDIZATION BETWEEN TWO SPECIES OF *PSEUDOPHRYNE*
(ANURA: LEPTODACTYLIDAE) IN THE SYDNEY BASIN,
AUSTRALIA

DAVID S. WOODRUFF*

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Synopsis

The red-crowned toadlet, *Pseudophryne australis*, is restricted to sandstone areas near Sydney. *P. bibronii* is more wide-ranging in eastern Australia, but is rarely found in the sandstone country because the ponds there typically dry out before larval development can be completed. Interspecific differences in coloration, adult size, breeding season and behaviour, and in rates of embryonic and larval development are documented.

Differences in habitat and breeding season reduce the possibility for interspecific contact. However, in situations where *P. bibronii* ranges into the sandstone country, occasional parapatric hybridization may occur at disturbed sites during the autumn. One population, which is intermediate between these two species in external coloration, size and shape, ovidiameter, egg number, and testes colour is described. Among the presumed hybrids, colour and morphometric characters vary independently. While hybridization may also account for colour intermediates found elsewhere, the possibility of convergence is considered.

INTRODUCTION

Two species of *Pseudophryne* occur in the Sydney Basin of eastern New South Wales. The red-crowned toadlet, *P. australis* Gunther, has conspicuous red patches on the top of the head and back. *P. bibronii* Gunther, in contrast, is dark grey or brown dorsally and lacks conspicuous red markings. The two species also differ in other aspects of coloration, size, breeding season and behaviour, and in rates of embryonic and larval development (Fletcher, 1889; Harrison, 1922; 1925; Moore, 1961; Jacobson, 1963*a*; 1963*b*; 1968; Woodruff, 1972). Most workers have interpreted these interspecific differences as adaptations to the contrasting habitats of the two species. *P. australis* occurs only in the Sydney Basin where it is restricted to areas in which Triassic sandstones outcrop. *P. bibronii* ranges widely over eastern Australia; and around Sydney it is typically found in areas where Triassic shale or Cenozoic sediments overlay the sandstone. While the present patterns of distribution correlate with surface geology closely, no detailed study on a microgeographic scale has yet been reported. Although Harrison (1922) concluded that the ranges of the two species were mutually exclusive, several lines of evidence indicate that interspecific contacts may occur in many areas. As Harrison noted, *P. bibronii* occasionally occurs on the tops of sandstone ridges, the normal habitat of *P. australis*. More recently, Moore (1961) found a roadside ditch which was occupied by *P. australis* from August to March, and by *P. bibronii* during April. In such areas, where differences in habitat cannot serve as premating isolating mechanisms, the possibility of interspecific interactions must be considered. Jacobson (1963*a*: 44) suggested (but provided no supporting data) that "Interbreeding may occur in nature. South of Sydney, where the habitats of these two species overlap, a few apparent colour intermediates have

* Department of Biological Sciences, Purdue University, West Lafayette, Indiana 47907 U.S.A.

been found". In this paper I provide additional details of the distribution of the two species and describe the nature of the interaction between them in parapatric situations.

METHODS AND MATERIALS

Coloration. General statements about coloration are based on the examination of all specimens in the collections of the Australian Museum, Sydney, and in the University of Melbourne Zoology Department up to July 1969. Statements about the coloration of specimens from areas of interspecific contact described in this paper are based on observations of living animals.

Size. Interspecific variation in ten characters was studied in samples of adult males. Insufficient numbers of females were available for comparative analyses; females are slightly larger than males. Measuring techniques and terminology are those of Woodruff (1975). No large samples were available to characterize each species; so after establishing that there was no significant ($p > 0.05$) intersample variation, several small samples were pooled. In the case of *P. australis* the pooled sample ($N=30$) included specimens from the Royal National Park, Lindfield Park and Pearl Beach. The sample used to characterize *P. bibronii* ($N=26$) included specimens from the You Yangs and Kerrisdale in Victoria, and Tarcutta, N.S.W. Museum specimen numbers and original data were reported by Woodruff (1972).

In the absence of a large sample of male *P. bibronii* from near Sydney, the use of data from other areas of south-eastern Australia can be defended on two grounds. First, a sample of 11 male *P. bibronii* from Lindfield was not significantly different from the pooled sample with respect to the first four variables in Table 2. (Comparable data were unavailable for the other variables.) Second, in a wider context, no significant variation was found in any of the 10 measures between the pooled sample used here and those of smaller samples from six other localities throughout the range of the species (Woodruff, 1972).

Distribution and Natural History. Distribution maps are based on the examination of all specimens in the collections of the Australian Museum and in the University of Melbourne up to July 1969. Published records and specimens in the Field Museum of Natural History, the Museum of Comparative Zoology at Harvard University, and the Museum of Vertebrate Zoology at Berkeley, do not extend the ranges of these species as determined by the two Australian collections. In addition, first hand observations on distribution and natural history were made in the course of four brief trips to the region during the period 1966–1969. Specimens collected on these trips were deposited in the University of Melbourne Zoology Department collection. Methods of handling batches of field-collected eggs are described by Woodruff (1976a).

COMPARATIVE MORPHOLOGY

Except in the case of specimens of intermediate coloration from a few areas, discussed below, the differences between *P. australis* and *P. bibronii* are marked and consistent (Table 1). Similarly, the morphometric data indicate that there are consistent differences in the mean size of adult males of the two species (Table 2). *P. bibronii* is generally larger-bodied than *P. australis*: the differences in adult male body length are particularly noteworthy. *P. australis* has relatively longer legs, feet and toes. These differences are accentuated in a comparison (mean and range) of the tibia length to body length ratio for these samples:

<i>P. australis</i>	0.34	(0.30–0.38)
<i>P. bibronii</i>	0.28	(0.25–0.32)

TABLE 1

P. bibronii—*P. australis* character index based on coloration of adult male specimens from the Sydney Basin

Character	Score	Remarks
Dorsum	0	Uniform dark brown or black as in <i>P. bibronii</i>
	1	Intermediate : few small orange or red patches
	2	Numerous small red patches on black background as in <i>P. australis</i>
Top of head	0	Dark brown or black as in <i>P. bibronii</i>
	1	Intermediate : dark with tiny red spots between the eyes
	2	Large red patch as in <i>P. australis</i>
Dorsal surface of upper arm	0	Inconspicuous brown or yellow patch or no patch as in <i>P. bibronii</i>
	1	Intermediate : orange patch of variable size
	2	Intermediate : red patch of variable size usually large
Post-femoral area	3	Conspicuous large white patch as in <i>P. australis</i>
	0	Inconspicuous brown or dull yellow patch as in <i>P. bibronii</i>
	1	Intermediate red patch of variable size
Supraclaoal area	2	Conspicuous large white patch as in <i>P. australis</i>
	0	Either uniformly dark, or with an inconspicuous dull yellow-brown spot or line extending across the back of the thighs as in <i>P. bibronii</i>
	1	Intermediate : Supraclaoal mark red or orange
Coccygeal mark	2	Uniformly dark as in <i>P. australis</i>
	0	Absent or a fine brown line as in <i>P. bibronii</i>
	1	Conspicuous red stripe as in <i>P. australis</i>

The data presented here are in close agreement with the findings of earlier workers who measured smaller samples and considered only one or two variables.

Although the results of parametric statistical tests are shown in Table 2, the assumption of normal distributions may be relaxed without affecting the conclusions reached. For example, a Mann-Whitney *U*-test on the tibia length data gave: $U=2660.90$, $z=19.26$; indicating that the null hypothesis (that the tibia lengths are the same in these samples) should be rejected.

Despite these statistically significant interspecific differences, it should be noted that it may be difficult to assign a colourless specimen to either species on the basis of one or two measures alone. Taxonomic decisions should be based on the examination of living animals or on a combination of several morphometric measures, as the diagnostic colour characters are alcohol-soluble.

DISTRIBUTION

P. australis is restricted to a small area of central coastal N.S.W. known as the Sydney Basin (Nashar, 1967). Specimens were collected at about 30 localities (Fig. 1). Locality records (excluding those published by Parker, 1940; and Moore, 1961) are: *Australian Museum*: Waterfall; Chatswood; Manly; Bobbin Head; Jaffa Ashord Downs; Narrabeen; *University of Melbourne Zoology Department*: 2.4 km N of Bilpin; Flat Rock Creek, Royal National Park; 0.6 km N of Menai; Castlecrag; Lindfield Park; Rocky Creek, St. Ives; Pearl Beach; 0.3 km N of Pacific Highway on Old Gosford road, between Pacific Highway and Somersby; 1.3 km N of Pacific Highway on Old Gosford road; Wishing Well State Forest, 7.3 km W of Cooranbong about 16 km W of Morisset. Specimen numbers and other data are given in Appendix A of Woodruff (1972).

The southern limit of the known range is Burrawang, while the western limits are near Leura and Mt. Irvine in the Blue Mountains. The northern limits were reported by Moore (1961) as Gosford and Kurrajong Heights, but I collected this species near Morisset about 40 km further north. Within this area the species has been found only where the Hawkesbury sandstone outcrop. It has not been collected in areas where shales of the Liverpool subgroup lie at the surface nor in areas covered by Tertiary or Quaternary deposits. If the distribution of Hawkesbury sandstone outcrops is an indication of the possible distribution of *P. australis*, then its range may be extended to the north-west into the rugged country between Kandos and Cessnock.

In addition to the localities reported here, there are a number of non-localized literature records of this species. Moore's (1961) reference to one such specimen in the Australian Museum (R8466) could not be confirmed but three specimens of this species are registered (R8468) without locality data. Also in the Australian Museum collection is a single specimen (R12418) referred to this species from Wide Bay, Queensland. As the specimen could not be located, its identity must remain conjectural. *P. australis* is not known from Queensland (Woodruff, 1976c).

P. bibronii is widespread in eastern Australia, ranging over 1600 km, from southern Victoria to south-eastern Queensland. If specimens of *Pseudophryne* from North Queensland described by Woodruff (1976c) are also referable to this species, then the range will extend nearly 2400 km in a north-south direction. Near Sydney specimens were collected at 40 localities (Fig. 2). Locality records (again excluding those published by Moore, 1961) are: *Australian Museum*: Burrawang; Oberon; Kanangra Walls; 8 km E of Guerie; Bexley North;

TABLE 2
Interspecific variation in size and shape of adult males

Character	Mean	Range	S.D.	t-test P	F-test P
Body length	20.58 24.69	(18.0—24.2) (22.0—28.0)	1.37 1.51	<<0.001	0.314
Tibia length	7.07 6.88	(6.5—7.5) (6.0—8.0)	0.20 0.43	0.037	<0.001
Head width	6.64 7.16	(5.7—7.5) (6.4—8.1)	0.44 0.37	<0.001	0.818
Head length	5.04 5.49	(4.5—5.7) (5.0—6.2)	0.36 0.37	<0.001	0.463
Toe 1 length	1.02 1.15	(0.8—1.2) (1.0—1.4)	0.10 0.10	<0.001	0.444
Toe 2 length	1.58 1.86	(1.3—1.9) (1.5—2.2)	0.11 0.19	<0.001	0.002
Toe 3 length	2.54 2.89	(2.0—3.0) (2.4—3.6)	0.22 0.26	<0.001	0.226
Toe 4 length	4.65 4.82	(4.0—5.0) (4.1—5.6)	0.26 0.42	0.038	0.003
Toe 5 length	1.61 2.00	(1.4—1.9) (1.6—2.3)	0.15 0.18	<<0.001	0.158
Foot length	6.91 7.17	(6.1—7.7) (6.4—8.3)	0.35 0.55	0.037	<0.001

Key: 1. In each couplet *Pseudophryne australis* (N=30) precedes *P. bibronii* (N=26).

2. Data are in mm.

3. The t-test indicated the probability that the means of the two samples are not significantly different if the variance ratio (F) test indicates that the two variances do not differ significantly.

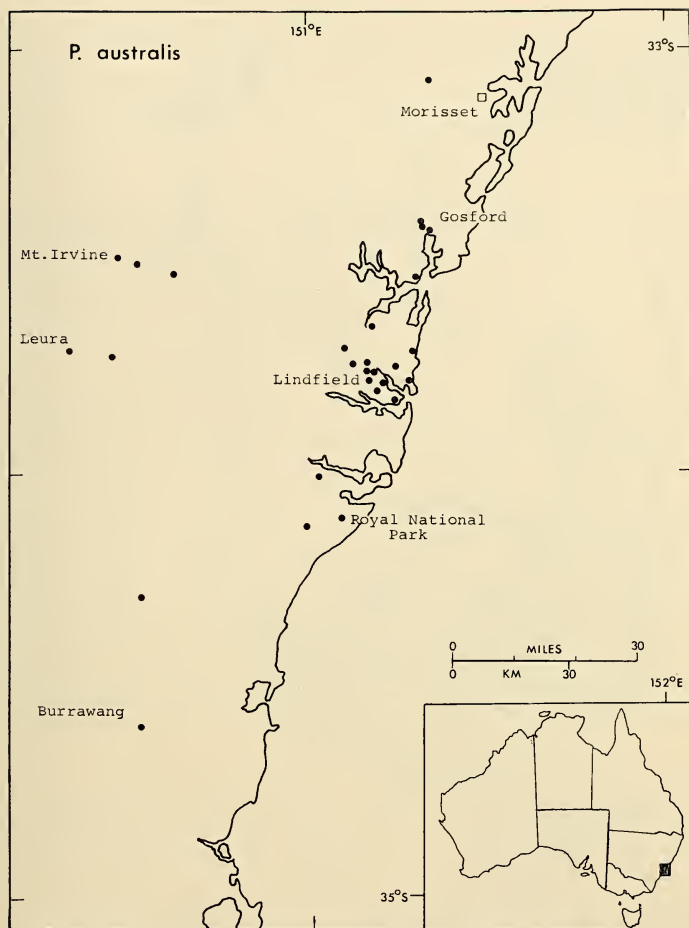


Fig. 1. Distribution of *Pseudophryne australis*. The position of the larger map is indicated in the inset.

Baulkham Hills; Ourimbah; *University of Melbourne Zoology Department*: Porters Retreat, Oberon; Lucas Heights; 0.4 km N of Menai; Thorpes Meadow, Menai; 4.8 km S of Wisemans Ferry; 16 km N of Windsor; 2.6 km and 3.4 km N of Pacific Highway on Old Gosford road, between Pacific Highway and Somersby; Mann's road, 2.3 km N of Gosford; 1.6 km N on Alford's Point road, Menai; 0.8 and 2.7 km N of Wyee; 4.3 km W of Cooranbong; Newcastle Forest Station, 10.6 km W of Cooranbong. Again, specimen numbers and other data are provided elsewhere (Woodruff, 1972).



Fig. 2. Distribution of *Pseudophryne australis* and *P. bibronii* near Sydney. Triassic shale outcrops in the shaded areas; Triassic Hawkesbury sandstone and more recent sediments outcrop elsewhere. Geological data from Department of Mines, N.S.W., 1 : 250,000 Geological Series, Sydney and Wollongong Sheets.

In the Sydney Basin the range of *P. bibronii* is fragmented, with isolated populations on residual outcrops of shale surrounded by sandstone. *P. bibronii* has also been collected on sandstone at Mosman, Lucas Heights, Menai, Killara and Gosford. This fact, coupled with the distribution of the two species (Fig. 2), suggests that the two taxa come into contact in many areas. Both species have been recorded from Burrawang, French's Forest, Gosford, Killara, Lindfield, Manly, Mosman and Wishing Well State Forest (about 16 km W of Morisset).

COMPARATIVE DEVELOPMENT BIOLOGY

External development has been described by Jacobson (1963*b*, 1968) and Woodruff (1972). Jacobson found that at 18°C *P. australis* developed rapidly, hatching after 26 days when the hind limb buds appear (stage 28 of Gosner, 1960) and metamorphosing after about 60 days. In contrast, she concluded that *P. bibronii* developed more slowly: hatching after 9 days, soon after the heart begins to beat, and metamorphosing after about 132 days. My studies of the development of these taxa confirmed her findings with respect to *P. australis*, but gave a different result in the case of *P. bibronii*. Briefly, I studied approximately 3000 *P. bibronii* eggs from the Sydney Basin area and found that at 16°C hatching occurs at stage 27-8 after about 30 days and metamorphosis occurs after 130-180 days. My results, which are supported by Moore's (1961) observations, suggest that while the encapsulated embryonic phase of development is similar in the two species, free-swimming larval development proceeds much faster in *P. australis* than in *P. bibronii*.

The discrepancy in our findings with respect to length of the embryonic phase in *P. bibronii* merits further comment here as the alleged differences in rates of development between these taxa have been discussed by other workers (Stephenson 1965; Salthé and Mecham 1974). Jacobson's observation of premature hatching in *P. bibronii* (actually her observations are referable to the closely related *P. dendyi*) may be attributable to thermal stress and/or abnormal encapsulation (a not uncommon phenomenon among *Pseudophryne* eggs laid in the laboratory). The embryos were maintained some 8-10°C above the mean temperature to which they would have been exposed in nature. In *Rana* spp. culturing under such conditions results in earlier hatching at earlier developmental stages (Yanai, 1952; Grainger, 1959).

COMPARATIVE REPRODUCTIVE BEHAVIOUR

Breeding sites. Both species lay their eggs in batches in nest sites on land. The larval phase of development is fully aquatic and of variable length, being about a month in *P. australis* and 3-5 months in *P. bibronii*. Eggs are laid in nest-sites which will become flooded so as to permit larval development. *P. bibronii* typically nests among the grass roots in marshy situations and in historical times has come to be associated with drains, roadside ditches and the edges of canals and dams. Such sites become flooded during the autumn and typically remain so until they dry out in the following summer. In the Sydney Basin such sites occur in areas where Wianamatta shale or Cenozoic deposits lie at the surface and are generally absent in the precipitous Hawkesbury Sandstone country. *P. australis*, in contrast, typically breeds in the damp leaf-litter along the course of first and second order streams and drains, and among the vegetation in the soaks that form on sheltered rock ledges in the Hawkesbury sandstone country.

Breeding season. While *P. bibronii* breeds only in the autumn in southern and eastern Australia (Woodruff, 1972; Fletcher, 1889), *P. australis* is widely believed to breed opportunistically throughout the year (Harrison, 1922; Jacobson, 1963*a*). Unfortunately, in reporting breeding activity few workers have made the distinction between male calling and actual mating and egg-laying

behaviour. In Victoria, where I studied the breeding behaviour of *P. bibronii* during five breeding seasons, males were heard calling from March 16–May 31 but egg laying did not commence until after April 4. Around Sydney, Fletcher (1889) and Harrison (1922) reported breeding activity during April, May and June and I have observed egg laying as late as June 15. The evidence that *P. australis* breeds throughout the year is not completely satisfactory. I agree that this species may call throughout the year but have reservations about Harrison's (1922) statement that it also mates in every month of the year. There is unequivocal evidence for mating in the months of August–January only (Fletcher, 1889, but see comments of next author on the specific identity of ova found in May; Harrison, 1922; Moore, 1961; Woodruff, 1972). I observed seven choruses in May and June but failed to find any females or eggs associated with them. Clearly the temporal aspects of this species' breeding season need clarification.

Breeding behaviour. Males of *P. bibronii* are territorial during the breeding season, establishing and maintaining exclusive calling areas centred around nest-sites (Woodruff, 1972; 1976b; and in preparation). Choruses contain 20–100 males and individual calling males were rarely found within 25 cm of one another. Several weeks after calling commences the females enter the area and mate with one or more males. Less than half the females lay all their eggs at once; most lay decreasing numbers of eggs at two or three successive matings over a period of several days (Woodruff, 1976b). Typically, 70–110 ova (ovidiameter: 1.6–2.1 mm) are produced at the initial mating. After laying all their eggs (up to 200) the females leave the breeding areas. Males, in contrast, typically remain with the eggs and resume calling. The nature and significance of this postmating brooding behaviour is discussed elsewhere (Woodruff, 1977).

Less is known about the breeding behaviour of *P. australis*. Harrison (1922) found males calling from hidden nest-chambers at a breeding site in Mosman. He reported that before oviposition both males and females occupy the nest, but after the eggs are laid the nest is deserted or the female alone remains. The eggs are usually laid at night after rain and, as many nests contained only a few eggs, Harrison speculated that the full complement of 20 eggs (ovidiameters: 2.6–3.5 mm) are produced over a period of four or five nights. Subsequently it was shown that a female can lay all her eggs at a single mating (Moore, 1961; Jacobson, 1963a).

The species of *Pseudophryne* differ from most other genera of anurans in that their conspicuous and most distinctive acoustical signal is essentially the same in all those species in which it has been studied quantitatively: *P. semimarmorata* (Littlejohn and Martin, 1969), *P. bibronii* and *P. dendyi* (Littlejohn, personal communication; Pengilley, 1971), *P. corroboree* (Pengilley, 1971). The calls of *P. australis* and *P. bibronii* cannot be distinguished by the human ear and preliminary audiospectrographic analyses have failed to reveal any conspicuous interspecific differences in call structure (Littlejohn, personal communication; personal observation). Littlejohn (1963, and subsequently) and Pengilley (1971) interpret the usual call made by *Pseudophryne* males in breeding choruses as the mating call. In contrast my observations (Woodruff, 1972) suggest that this call functions primarily in the establishment and maintenance of male breeding territories. Regardless of whether the common vocalization is more correctly termed a mating call or a territorial call, there is no evidence to suggest that it could serve as a premating isolating mechanism between the two species.

In conclusion, it would appear that where the ranges of these two species contact during March–June there will be few premating barriers to interspecific hybridization.

KNOWN INTERSPECIFIC CONTACTS

Killara. Moore studied *P. australis* in the ditch along the St Ives Road in Killara. This site was occupied from 3rd August 1952 until 12th March 1953; "Then for a period of a month, which was very dry, the site was not visited. On April 13 and 14 there was rain, and on April 15 the site was checked. *Pseudophryne* males were calling, and with the removal of about an inch of mud, four nests were found, but the frogs with them were *Pseudophryne bibronii*. No *Pseudophryne australis* were found" (Moore, 1961: 236). I searched in the few remaining ditches along St Ives Road in June, 1967 but no *Pseudophryne* were found. Attempts to locate *Pseudophryne* in other areas of possible inter-specific contact in the suburbs of Sydney (Manly, Lindfield, Mosman) also proved unsuccessful.

Wishing Well State Forest. In the hills about 16 km W of Morisset, at the northern limit of the known range of *P. australis*, the two species apparently are sympatric. I collected a juvenile *P. australis*-like toadlet in the forest 7.3 km W of Cooranbong on 15th June 1967. Jacobson's (1963*b*) work on rates of development suggests that in this area *P. australis* bred six months earlier. On 22nd May 1969 I found an adult *P. bibronii* within 0.3 m of the initial collection site. Although no other *Pseudophryne* were found at this site on either occasion specimens were collected at two nearby sites on 23rd May 1969. Four male *P. bibronii* found at 4.2 km W of Cooranbong had unusual red patches on the upper surface of their arms, thighs and dorsum. Two of these males were attending batches of 37 and 38 eggs respectively. The embryos developed normally, hatching at stage 26-27 and experiencing 5.4 and 2.6% embryonic mortality. Another male *P. bibronii* was collected 10.6 km W of Cooranbong and had normal pigmentation. This animal was associated with 79 eggs which hatched at stage 27 and experienced 4.1% embryonic mortality. Embryonic mortality in *P. bibronii* is typically less than 5% (range 0-11%) (Woodruff, 1976*a*).

Gosford. *P. australis* occurs in the sandstone country around Gosford, and *P. bibronii* occurs on the hills to the north-west of the town. On the Old Gosford road (which climbs from the Pacific Highway in the valley 2.5 km W of Gosford to Somersby, 6.5 km to the north), the two species were found within 1.3 km of one another. On 21st-22nd May 1969, *P. australis* was collected at sites 0.3 and 1.3 km N of the Pacific Highway; *P. bibronii* was found 2.6 and 3.4 km N of the same highway. Sandstone outcrops at the three localities closest to the highway. The two specimens of *P. bibronii* collected on sandstone, 2.6 km N of the highway, had unusual red spots on the dorsum and across the back of the thighs. A batch of 110 eggs collected with one of these males hatched at stage 27 and showed very high (40%) embryonic mortality. 0.8 km further north on the Old Gosford road four typical *P. bibronii* were found together with two batches of unattended eggs. Both batches were typical of *P. bibronii* comprising 68 and 377 embryos that hatched at stage 27 and exhibited 1.4 and 4.0% embryonic mortality respectively. (See Woodruff (1976*b*) for a discussion of the large batch: a result of multiple mating at a single nest-site.) At the time these observations were made *P. bibronii* were calling and mating in this area; *P. australis* were not breeding; males were solitary and hidden beneath leaf litter away from the presumed breeding sites.

Menai. Near the village of Menai, 24 km S of Sydney, I discovered a population exhibiting far greater colour intermediacy than any previously reported. The area is shown in Figure 3. *P. bibronii* occurs on the shale capping at Menai (Thorpe's Meadow, Locality E), and on a sandstone ridge (Localities D, B) that runs north towards Alford's Point. *P. australis* also inhabits this ridge. At Locality A on 27th May 1968 a large sample of *Pseudophryne* (51 males, 2 females) with intermediate coloration was obtained from beneath

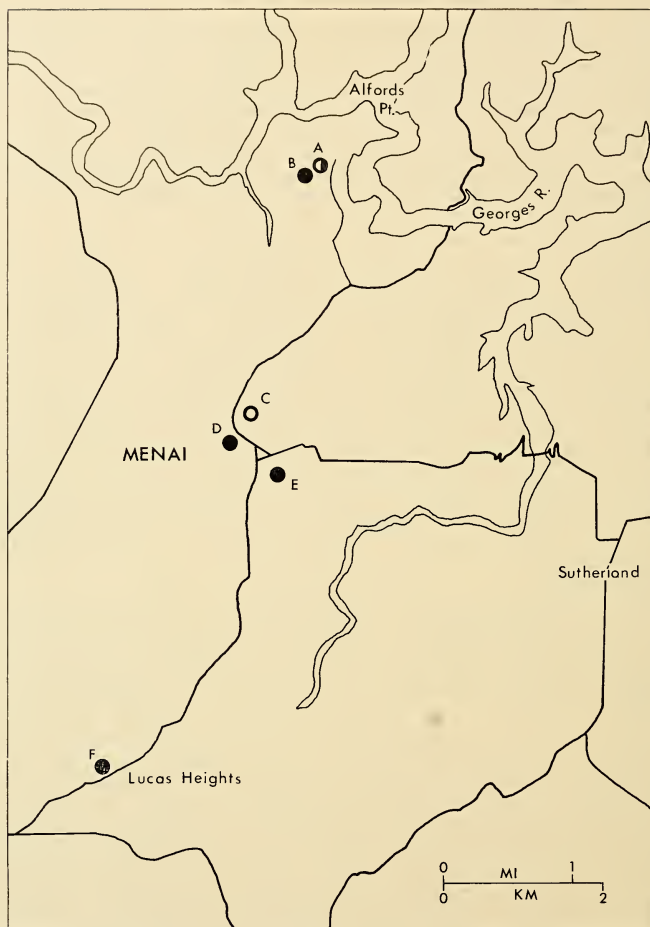


Fig. 3. Distribution of *P. australis* (open circle), *P. bibronii* (closed circles), and an intermediate population (half-closed circle) near Menai. Locations A-E are discussed in the text.

rocks around a small shallow pond. The site was revisited on 21st May 1969 when five *P. bibronii*-like males were found. On that occasion two male *P. bibronii* were found in a shallow depression about 100 m further east (Locality B). In the following detailed analyses the two samples from Locality A at Alford's Point will be referred to as AP-1968 and AP-1969 respectively.

THE ALFORD'S POINT CONTACT

Coloration. All 53 specimens in the AP-1968 sample were scored (Table 3,4) using the character or hybrid index system (Table 1). 75% (40/53) of the toadlets have coloration atypical of *P. bibronii* and tending towards that of *P. australis*. 51% have some *P. australis*-like red markings on the dorsum; 6% have *P. australis*-like red patches on the head; 40% have upper arm patches that are atypical of *P. bibronii* (in either size or colour); 6% have unusual patches in the post-femoral area; 36% have supraclacal markings that are atypical of *P. bibronii*; and one animal has a broad *P. australis*-like coccygeal stripe.

In contrast to the pattern of colour intermediacy described above, the AP-1969 sample comprised five specimens of typical *P. bibronii* with character indexes of zero. The two males collected nearby (Locality B) at the same time were also very close to typical *P. bibronii* having character indexes of two (presence of large red arm patches). Similarly 27 of the remaining 29 *P. bibronii* collected at Localities D and E showed no atypical coloration; the exceptions were two males with large arm patches from Thorpe's Meadow (character indexes =1, 2).

TABLE 3

Summary of the variation in coloration of 53 *Pseudophryne* specimens collected at Alford's Point (Locality A) in 1968

Character	<i>P. bibronii</i> -like	Intermediate	<i>P. australis</i> -like
Dorsum	26	13	14
Head	50	2	1
Upper arm	32	21 (11+10)	0
Post-femoral mark ..	50	3	0
Supraclacal mark ..	34	10	9
Coccygeal mark ..	52	—	1

Morphometrics. The adult males in AP-1968 and AP-1969 were measured for the same ten variables that were employed in the analysis of interspecific differences presented above (Table 5). It is difficult to see the overall pattern in such a matrix, but mean values for AP-1968 lie between those of *P. australis* and *P. bibronii* for seven variables out of ten. In the three remaining variables (tibia, head length, and toe 4 lengths) the AP-1968 sample mean value lies only slightly outside the range between the mean values of *P. australis* and *P. bibronii*. The univariate analysis suggests that the Alford's Point animals are of intermediate size and shape. The matrix of coefficients of variation (Table 6) shows that the level of variability for any individual character is roughly similar in all samples. Notably, the samples of intermediate morphology do not exhibit generally higher (or lower) levels of morphometric variability.

Such multivariate data are more appropriately treated by various multivariate statistical techniques; I performed a canonical analysis to establish a discriminatory topology for these four groups. The stepwise discriminant analysis involved the use of program BMD07M (Health Services Computing Facility, University of California, Los Angeles, 1973) and the matrix of factor patterns was calculated using program D/DA (John Rhoades, Harvard University).

The results of the multivariate discriminant analysis are shown graphically in Fig. 4. The group (sample) centroids and the 112 individual specimens are plotted against the first two canonical axes. The first canonical variate accounts for 74% of all information, the second for 20%, the third for over five of the remaining 6%. Projections on the first axis, the best multivariate discriminator

TABLE 4

Summary of the variation in coloration of 53 *Pseudophryne* specimens collected at Alford's Point (Locality A) in 1968

Character							
N	1	2	3	4	5	6	Index
13	B	B	B	B	B	B	0
7	I	B	B	B	B	B	1
4	A	B	B	B	B	B	2
4	A	B	B	B	I	B	3
3	B	B	I	B	B	B	1
2	B	B	B	B	I	B	1
2	B	B	A	B	B	B	2
2	A	B	I	B	B	B	2
1	B	B	I	B	I	B	2
1	I	B	I	B	B	B	2
1	I	B	A	B	B	B	3
1	I	B	B	B	I	B	2
1	B	B	I	B	A	B	3
1	B	A	B	A	B	B	3
1	A	B	I	B	I	B	4
1	I	B	I	B	A	B	4
1	B	B	A	B	A	B	4
1	A	I	I	B	I	B	5
1	I	B	A	B	A	B	5
1	B	B	A	A	A	B	5
1	I	B	A	B	A	A	6
1	A	B	A	B	A	B	6
1	A	I	A	B	A	B	7
1	A	B	A	A	A	B	7

- Key: 1. The number of specimens (N) with each combination of character states are indicated.
 2. Characters are as described in Table 1 except that the two intermediate states of character 3 are grouped.
 3. B=*P. bilronii*-like;
 I=intermediate;
 A=*P. australis*-like.
 4. The character or hybrid index is based on scores shown in Table 1.

TABLE 5

Mean values for *P. australis*, *P. bilronii*, and two samples from Alford's Point, in mm

Variable	<i>P. australis</i> N=30	AP-1968 N=51	AP-1969 N=5	<i>P. bilronii</i> N=26
Body length ..	20.58	22.13	24.24	24.69
Tibia length ..	7.07	6.83	7.48	6.88
Head width ..	6.64	6.79	7.02	7.16
Head length ..	5.04	5.58	5.61	5.49
Toe 1 length ..	1.02	1.10	1.03	1.15
Toe 2 length ..	1.58	1.63	1.71	1.86
Toe 3 length ..	2.54	2.54	2.64	2.89
Toe 4 length ..	4.65	4.62	4.90	4.82
Toe 5 length ..	1.61	1.67	1.63	2.00
Foot length ..	6.91	6.93	7.32	7.17

among groups, displays the expected pattern of intermediacy in the Alford's Point samples. The morphometric positions of the AP-1968 and AP-1969 group centroids lie almost exactly halfway between the group centroids of the homospecific samples (high projections) and the Alford's Point samples (low projections). Considering all axes simultaneously, Mahalanobis' generalized distances between the various group centroids can be calculated. The AP-1968 sample is again approximately equidistant from the homospecific sample centroids: D_1 (AP-1968 to *P. australis*) = 2.78 : D_2 (AP-1968 to *P. bibronii*) = 3.28.

The matrix of factor patterns (Table 7) gives the correlations between the ten original variables and the three main discriminant functions; from it we can assess the patterns of covariance that determine differences among samples. Body length and toe 5 length have high correlations with the first axis; these two measures vary independently of one another ($r=0.0962$). It may be recalled that these are the variables that had the highest F-ratios as univariate discriminators. Only one variable (head length) has a high correlation with the second axis and it too had a high F-ratio as a univariate discriminator. Body length and tibia length are highly correlated with the third canonical variate.

TABLE 6
Coefficients of variation for samples described in Table 5

Variable	<i>P. australis</i>	AP-1968	AP-1969	<i>P. bibronii</i>
Body length ..	6.66	4.65	8.54	6.12
Tibia length ..	2.83	5.27	4.95	6.25
Head width ..	6.63	5.89	9.12	5.17
Head length ..	7.14	5.20	8.38	6.74
Toe 1 length ..	9.80	11.82	5.83	8.70
Toe 2 length ..	6.96	8.59	14.04	10.22
Toe 3 length ..	8.66	7.87	6.44	9.00
Toe 4 length ..	5.59	6.71	4.08	8.71
Toe 5 length ..	9.32	9.58	3.68	9.00
Foot length ..	5.07	5.34	2.87	7.67

I conclude from this discussion that the Alford's Point samples are morphometrically intermediate between *P. australis* and *P. bibronii*. Still further support for their hybrid nature comes from the fact that they lie displaced to one side of the line in phenetic hyperspace that joins the two parents. Whitehouse (1969), working with strains of barley and beans, found that F_1 interstrain hybrids typically lay midway between their parents but displaced laterally about 35% of the interparental distance. Displacement was rather less for F_2 hybrids. In the present case the group centroids for AP-1968 and AP-1969 lie about halfway between the homospecific sample centroids and are offset 30-40%. Sneath and Sokal (1973) have reviewed some genetic and mathematical reasons for this displacement.

Coloration and morphometric considered simultaneously. It is pertinent to inquire whether there is any relation between a specimen's position in the discriminatory topology (Fig. 4) and its character index based on coloration. To answer this question the distance (in three-dimensional space) between each individual specimen and the *P. australis* group centroid was first calculated. Considering all 51 specimens, the correlation coefficient between distance (x) and character index (y) was 0.009. A straight line with a slope $y=2.017+0.20x$ was fitted to the data points by the least squares method. The coefficient of determination ($r^2=0.00008$) indicates that the regression line has very poor "goodness of fit". I conclude that measures of intermediacy based on coloration and morphometrics vary independently of one another.

Other aspects. All adults collected at Alford's Point appeared normal in health and behaviour. The collection of one male with a malformed foot is not judged unusual; I found such specimens at low frequency in samples from throughout the range of *Pseudophryne*. Internal anatomy of eight males and three females from AP-1968 was examined macroscopically. Testes were found to be more variable in both size and colour than similar samples of homospecific *Pseudophryne* from south-eastern Australia. Considerable variation was also

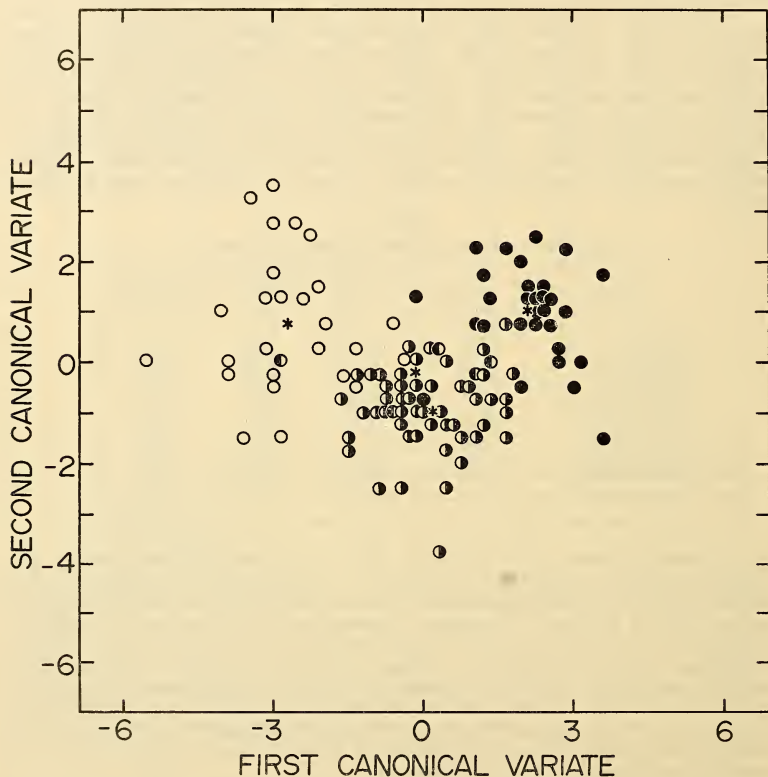


Fig. 4. Discriminatory topology for the four samples of *Pseudophryne* described in Table 5. Asterisks indicated sample centroids. The symbols indicate the positions of the individual specimens. Open circles represent specimens of *P. bibronii*; closed circles, *P. australis*; circles with right side closed, AP-1968; circles with left side closed, AP-1969.

found in the ova dissected from three females. One female contained 203 relatively small ova (mean ovidiameter: 1.88 mm; range: 1.7–2.0 mm), typical for *P. bibronii* from eastern New South Wales. In another female there were 47 ova (mean ovidiameter: 2.28 mm; range: 2.2–1.4 mm). In the remaining female a distinct size difference was noted within the group of 45 non-ovarian eggs: some were relatively small ($\bar{x}_{12}=2.27$ mm; range: 2.0–2.4 mm)

TABLE 7
Factor pattern for the first three canonical axes

Variable	Axis 1	Axis 2	Axis 3
Body length ..	0.6581	-0.0655	0.6035
Tibia length ..	-0.1009	-0.2428	0.7286
Head width ..	0.2626	-0.0879	0.1909
Head length ..	0.2590	0.5548	0.2188
Toe 1 length ..	0.2329	0.1017	-0.2204
Toe 2 length ..	0.3890	-0.2499	0.0982
Toe 3 length ..	0.3302	-0.3516	0.0311
Toe 4 length ..	0.1109	-0.1641	0.3034
Toe 5 length ..	0.5122	-0.3605	-0.2802
Foot length ..	0.1365	-0.1118	0.3558

while others were large ($\bar{x}_{15}=2.95$; range: 2.8-3.0 mm) like those of *P. australis*.

Two batches of eggs from Thorpe's Meadow (Locality E) hatched after about one month at stage 27 (at 16°C). Batch sizes and embryonic mortality (73; 8.2%; and 109; 4.6%) were characteristic of typical *P. bibronii*. Tadpoles developed slowly and two metamorphosed about five months after collection. Juvenile coloration was typical for *P. bibronii*. Batch size in the eight batches collected at the AP-1968 site ranged from 10-85 ($\bar{x}=48.3$) eggs. They were thus smaller than expected for *P. bibronii* (typically 80 eggs) in this area. Embryonic mortality was normal in six batches (0.0-6.8%), and slightly higher in two batches (16.6% in 72 eggs; and 40% in 10 eggs).

DISCUSSION

There are at least two explanations for these observations and at the outset it should be emphasized that they are not mutually exclusive. First, as Jacobson (1963a) suggested, interspecific hybridization may be occurring in some areas. Hybridization could account for the intermediate coloration of *P. bibronii* in Wishing Well State Forest, Gosford and Menai. Variation in coloration of the presumed hybrids is similar in many respects to that seen between the various subspecies of the salamander *Ensatina eschscholtzii* (Stebbins, 1949; Brown, 1974), and between the various colour morphs of *Salamandra salamandra* and *Ambystoma maculatum* (Noble, 1931). Hybridization could also account for the unusual nature of the testes and ova of AP-1968 specimens. The strongest evidence for this interpretation, however, is provided by the morphometric analysis. In this context it is worth mentioning that no special significance is attached to the lack of increased variability in the AP-1968 sample. While some workers (see Mayr, 1963, for a review) have held that hybrid populations are typically more variable than homospecific ones, there are many cases where this is not so (Simpson *et al.*, 1960; Sokal and Rohlf, 1969). More recently Endler (1977) has shown that, despite longstanding claims to the contrary (see, for example, Mayr, 1963), levels of variation cannot be employed to distinguish zones of primary hybridization from zones of secondary hybridization.

An alternative explanation of the unusual coloration of the specimens of *P. bibronii* found on sandstone at the sites described above is that they represent the products of convergent evolution. Thus, the red markings and smaller egg batch size, which are presumably at a selective advantage in *P. australis*, may also be selected for in *P. bibronii* living in the same habitat. While I favour the hypothesis of interspecific hybridization in the case of the Alford's Point samples, I cannot exclude the possible effects of convergent evolution either in this case or at the other two sites I have studied.

The problem of *P. australis* coloration has several aspects which will be discussed here as they have a bearing on the hypothesis that the orange and red marks on the dorsal surface of *P. bibronii* in this area evolved by convergence. First, it should be remembered that organisms that lack colour vision probably perceive the red patches only as dark grey markings on a black background. In their case the large white patches on the upper arms and thighs of *P. australis* may be far more conspicuous. These patches tend to disrupt the outline of the animal's body against its background. Similar patterns are seen in *P. dendyi* and *Kankanophryne occidentalis*, and are most highly developed in the cryptic *P. corroboree* (Woodruff, 1972; 1975). Elsewhere in the Amphibia a strikingly similar case is described by Stebbins (1949) and Brown (1974). The red or orange patches on the dorsal surface of *P. australis* will, however, be perceived as such by a variety of diurnal animals including some insects, reptiles, birds and primates. The coloured markings could serve two purposes in this context. First, the markings disrupt the outline of the animal in the leaf litter and should contribute significantly to the animal's overall crypsis if it is uncovered during the day. Second, it is possible that *P. australis* is a mimic in a Batesian mimicry system; and that its red patches are modelled on similar coloration in some distasteful or obnoxious organism. The only candidate for the model I encountered was the cockroach *Polyzosteria limbata* Burmeister, which when disturbed raised its wings and abdomen, exposing striking yellow-orange markings beneath. These insects were common enough among the leaf litter to serve as a model for non-breeding (dispersed) *P. australis*. This species is presumably protected by abdominal stink glands as are many other cockroaches. H. G. Cogger, to whom I am indebted for the identification, advises that this insect extends from the Clarence River to Mallacoota, and as far west as Orange, but appears to be most abundant in the Sydney region (Cogger, *in litteris*, 1976).

Clearly, additional field work will help resolve the various questions raised in this paper. In this connection several points must be made. First, it is most unlikely that short-term studies of the type made by Moore and myself will answer these questions. The change in the coloration of the Alford's Point population between sample AP-1968 and AP-1969 indicates the importance of planning studies of several years duration. It is quite possible that interspecific hybridization, if it does occur, occurs on a limited basis. As Loftus-Hills (1975) has argued for the case of *Bufo americanus* and *B. woodhousii*, environmental conditions may be such that there is no overlap in breeding season in some years. In this case one would expect considerable variation in the frequency of hybridization over a period of a few years. The same may apply to *P. australis*-*P. bibronii* and underscores the need to define more precisely the breeding seasons of these two species in the Sydney Basin area. Another point that must be borne in mind in planning further studies on this problem is that hybridization is probably restricted to very localized areas. Available data suggest that it is typically *P. bibronii* that is encroaching into the range of *P. australis*. Parapatric hybridization (*sensu* Woodruff, 1973), when it occurs is probably restricted to a very narrow zone. The role of man in increasing the possibilities for interspecific contact in historical times must also be kept in mind. All the sites of contact described here have been altered by human activities.

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